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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

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JUN 6 1975

Application for Research Grant

(Use extra pages as needed)

Date: May 29, 1975

1. Principal Investigator (give title and degrees):

Lee W. Wattenberg, M.D. - Professor of Pathology

2. Institution & address:

University of Minnesota
Medical School
Department of Laboratory Medicine/Pathology
184M Jackson Hall
Minneapolis, MN 55455

3. Department(s) where research will be done or collaboration provided:

Laboratory Medicine & Pathology

4. Short title of study:

Inhibition of Carcinogenesis by Benzyl Isothiocyanate and Related Compounds.

5. Proposed starting date: 10-1-75

6. Estimated time to complete: Three years

7. Brief description of specific research aims:

1. To determine conditions under which benzyl isothiocyanate and related compounds will inhibit neoplasia resulting from exposure to polycyclic aromatic hydrocarbons.

2. To determine the capacity of these compounds to inhibit neoplasia due to other classes of carcinogens.

3. To determine the mechanism(s) of inhibition .

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8. *Brief statement of working hypothesis:*

Experiments have shown that benzyl isothiocyanate will inhibit BP-induced neoplasia of the stomach and lung of the mouse and DMBA-induced mammary tumor formation in the rat. The mechanism(s) of this inhibition is not known nor have the conditions under which it occurs been defined. Inhibition may be due to direct interaction of benzyl isothiocyanate with reactive metabolites of the carcinogens. An alternate possibility is that the metabolism of the carcinogens have been changed by an effect of benzyl isothiocyanate on the enzymes carrying out these reactions. Both possibilities will be explored.

9. Details of experimental design and procedures (append extra pages as necessary)

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Background Information. Studies which we have carried out recently have shown that benzyl isothiocyanate and benzyl thiocyanate have the capacity to inhibit neoplasia resulting from exposure to polycyclic aromatic hydrocarbon (PAH) carcinogens (1). These compounds are primarily constituents of cruciferous plants, but are found in several other plant families as well. Of the two compounds, benzyl isothiocyanate is more potent and more versatile as an inhibitor of chemical carcinogenesis than the corresponding thiocyanate, so that much of the initial work centers on this compound. Benzyl isothiocyanate will inhibit mammary tumor formation in the rat when administered by oral intubation or intraperitoneally four hours prior to administration of a carcinogenic dose of DMBA. If benzyl isothiocyanate is added to a diet containing benzo(a)pyrene (BP) it will inhibit the occurence of tumors of the forestomach and lung resulting from this carcinogen. Benzyl thiocyanate will inhibit DMBA-induced mammary tumor formation but thus far has not been found to be effective in suppressing formation of gastric or pulmonary tumors. Sodium thiocyanate is inactive as an inhibitor in all systems which have been studied. The mechanism(s) by which benzyl isothiocyanate inhibits neoplasia is not known. Of possible significance are biochemical studies which have shown that if this compound is added to a microsomal system in vitro, AHH activity is inhibited.

Effect of benzyl isothiocyanate on the nature and quantity of metabolites of BP formed by the microsomal mixed function oxidase system. The initial studies that we proposed carrying out will be directed at determining possible quantitative and qualitative differences in metabolites which occurs when BP is incubated with microsomes from lung, liver or squamous stomach of mice fed

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benzyl isothiocyanate as compared to corresponding controls. High pressure liquid chromatography (hplc) will be employed following recently described procedures (2,3). This technique results in a separation of a large number of metabolites, including phenols, diols, and quinones. Thin-layer chromatography using tritium-labelled BP will be employed as an adjunct to the work with hplc. In results with hplc presented thus far, the presence of 6-OH-BP and BP epoxides have not been reported. It is likely that these compounds are too reactive to survive the workup procedures and high temperature conditions (50°) of the hplc techniques used thus far. To trap these highly reactive species, we plan to employ low temperature extraction with the exclusion of light and oxygen and to use ambient temperature hplc. In addition, efforts will be made to form derivatives of the metabolites with various silylating agents. These silyl derivatives will be analyzed by glc and mass spectroscopy. An alternative possibility for studying 6-OH-BP is to employ the method of Ts'o et al (4,5). Depending upon the results obtained, additional analytical procedures or modifications of the procedures described will be employed.

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Effects of benzyl isothiocyanate on binding of BP metabolites to DNA. Current evidence suggests that the extent of macromolecular binding of carcinogen metabolites is a critical parameter for determining the neoplastic potential of the carcinogen under investigation. Accordingly, it would be of importance to determine to what extent the magnitude of DNA binding is altered by administration of benzyl isothiocyanate under conditions in which it inhibits chemical carcinogenesis. The initial studies of this type will be carried out employing an experimental model in which benzyl isothiocyanate is fed at a dose level resulting in inhibition of neoplasia. Microsomes from lung, liver, and

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small bowel will be isolated. These will be used for studying DNA binding of BP metabolites under conditions where the parent carcinogen is incubated in the presence of liver microsomes, NADPH, and DPN. ^{14}C-labelled BP will be employed. Following incubation, the DNA will be isolated employing a modification of the procedure used by Grover and Sims (6).

Studies of DNA binding with BP-7, 8-dihydrodiol. Recent work has shown that incubation of liver microsomes with BP-7,8-dihydrodiol, a metabolite of microsomal metabolism of BP, results in a high degree of binding to DNA (7). In addition, studies by Brooks et al indicate that the bound product has characteristics similar to that of the bound metabolites of BP which are formed in vivo (8). These data suggest that one of the possible pathways by which BP becomes bound to DNA entails basically two metabolic cycles. In the first, BP is converted by microsomes to the 7,8-dihydrodiol. Subsequently, this metabolite is metabolized again by the microsomal mixed function oxidase system and this time is converted to BP-7,8-dihydrodiol,9,10-epoxide. This latter compound is highly reactive and binds to DNA. In the work proposed, we would like to determine whether the microsomes from benzyl isothiocyanate fed mice as compared to control mice differ in their capacity to convert the BP-7,8-dihydrodiol to the metabolite which binds to DNA. Reaction conditions comparable to those used for the DNA binding studies in which BP is employed will be utilized. Parallel studies will be run employing both BP and BP-7,8-dihydrodiol.

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Studies of epoxide metabolism. Epoxides are formed as intermediate products of BP metabolism and can react with cellular macromolecules such as DNA. Epoxide hydrase is a microsomal enzyme which converts epoxides to dihydrodiols. This constitutes an important step in the metabolism of BP as well as other polycyclic hydrocarbons. Accordingly, we propose to determine the effects of benyzl isothiocyanate administration on the activity of this enzyme. The procedure

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employed will be based on that of Oesch et al (9). Tritiated styrene oxide or benzo(a)pyrene-4,5-oxide will be used as substrates. Another pathway of epoxide metabolism entails conjugation with glutathione (glutathione S-epoxide transferase). This conjugation will be studied employing the procedure of Booth and Sims (10).

Studies of formation of BP conjugates. In addition to the experiments described above, procedures will be carried out to study glucuronide and sulfate conjugation of BP in benzyl isothiocyanate fed and control animals. Cell fractions from liver or forestomach of control and benzyl isothiocyanate fed mice will be incubated with BP or BP metabolites and added uridine diphosphate-D-glucuronic acid (UDPGA) or 3-phosphoadenosine-5'-phosphosulfate (PAPS). High pressure liquid chromatography in an ion-exchange mode will be used to study water-soluable conjugates from the above reactions. The metabolites of BP which will be used as substrates initially are 3-OH-BP, 6-OH-BP, and BP-7,8-dihydrodiol. The 3-OH-BP is a relatively stable metabolite which will be useful as a prototype of conjugation of a monophenol derivative. The 6-OH-BP is an unstable compound and reaction conditions for its use may be difficult to work out. However, it is an important compound being a possible proximate carcinogen, so that its rate of conjugation could determine the amound available for macromolecular binding. Accordingly, efforts will be made to establish a procedure which can quantitatively determine its conjugation. BP-7,8-dihydrodiol,9,10-oxide reacts with macromolecules. If BP-7,8-dihydrodiol is conjugated, the epoxidation would be prevented. Thus, this substrate is important to study because of its potential biological effects. It also represents a prototype of a dihydrodiol metabolite of BP. In later studies, other metabolites of BP will be employed as substrates. After the methodology has been established for identifying and quantitating the conjugates, studies of their biliary and urinary excretion in

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control and BHA fed mice given ^{14}C-BP will be attempted.

Studies of the reactions between benzyl isothiocyanate and metabolite(s) of BP in a microsomal system. Inhibition of polycyclic hydrocarbon induced neoplasia by benzyl isothiocyanate may possibly be the result of a reaction between a highly reactive metabolite or metabolites of BP and benzyl isothiocyanate. In essence, what would be occurring is that the isothiocyanate scavenges the reactive form of the carcinogen metabolite before it has a chance to bind to macromolecules. If this mechanism exists, it might be possible to demonstrate a reaction product between benzyl isothiocyanate and BP metabolite(s). Current evidence indicates that there are two principle types of highly reactive metabolites of BP which can bind to macromolecules. The first consists of a group of arene oxides and the second, positively charged radicals (5,11-14). We propose carrying out three types of experiments. The first will be studies of the reactions of reactive metabolites of BP belonging to these two groups with benzyl isothiocyanate in simple solutions. The second will be an effort at demonstrating the presence of reaction product(s) after incubation of BP and benzyl isothiocyanate in a microsomal system and the final study will be an effort demonstrating the occurance of reaction product(s) in vivo. For the initial studies of the interactions of active metabolites of BP and benzyl isothiocyanate in solution we propose to employ three BP derivatives. The first two are BP-4,5-oxide and BP-7,8-dihydrodiol,9,10-oxide. A great deal of evidence has implicated arene oxides as proximate carcinogens of BP (8,11,12). The evidence for BP-7,8-dihydrodiol,9,10-oxide being a proximate carcinogen has been presented previously. The third is the 6-oxo-BP radical which forms from 6-OH-BP (4,5). This is a highly reactive radical which binds to macromolecules. Studies of Ts'o and colleagues have shown that 6-OH-BP comprises about

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15% of the total metabolites formed on incubation of BP with liver microsomes (4). Thus from both qualitative and quantitative aspects, this compound is of considerable interest. The active metabolites of BP will be allowed to react with benzyl isothiocyanate both in aqueous and organic solvent systems and the products of these reactions isolated. Their structures and physical and chemical properties will be studied, including behavior in procedures such as high pressure liquid chromatography and thin layer chromatography which will be used in subsequent identification procedures in complex reaction mixtures.

The second group of studies will consist of incubations of ^{3}H-BP and ^{14}C benzyl isothiocyanate in a liver microsomal system. Possible interaction products along with the metabolites and starting materials will be extracted into organic solvents from the incubation mixture. High pressure liquid chromatography and thin layer chromatography will be used to separate possible interaction products from the rest of the metabolites and would be identified by the presence of both ^{3}H and ^{14}C radioactivities in appropriate ratios. The finding of such entities would be followed by efforts at large scale isolation and structural elucidation of reaction products. In order for this to be accomplished, isolation of workable quantities of pure materials are necessary. With such materials, physical data such as melting points; elemental analysis; spectral data including infrared, ultraviolet, and fluorescence; nuclear magnetic resonance; and mass spectroscopic analysis will be obtained for determination of chemical structure. These data will be compared with those of the chemically synthesized reference compounds. If a reaction product or products can be identified in an <u>in vitro</u> system, further work will be carried out in an effort to demonstrate the comparable material <u>in vivo</u>. This will entail administration of ^{3}H-BP and ^{14}C benzyl isothiocyanate to mice in subsequent attempts to establish the presence of the reaction product in tissues.

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Studies of conditions under which benzyl isothiocyanate will inhibit BP carcinogenesis. Work will be carried out with four animal model systems to determine conditions under which benzyl isothiocyanate will inhibit BP carcinogenesis. From these studies it should be possible to obtain information as to the effects of various routes of administration of inhibitor and carcinogen and also basic information on animal models most effective for additional studies. Such studies will include dose-response, those involving other compounds to be investigated for their inhibiting properties, and also work with additional carcinogens. The first animal model will entail the the use of Ha/ICR mice (15). These animals were employed in work already carried out which showed that addition of benzyl isothiocyanate to a diet containing BP will inhibit the formation of gastric tumors as well as pulmonary tumors. We propose to carry out two further studies with this animal model. In the first, the carcinogen will be given by oral intubation rather then added directly to the diet. This has the advantage of providing precise information as to the amount of carcinogen consumed. In a second modification carcinogen and benzyl isothiocyanate will both be given by oral intubation. The second set of studies will entail the use of A/HeJ mice. These animals have a high susceptibility to pulmonary neoplasia. A great deal is known about the response of this animal model to various carcinogens and factors which alter carcinogenicity. Thus it is a versatile system for studying inhibition of neoplasia. We propose carrying out a study in which the carcinogen is given by oral intubation and the benzyl isothiocyanate in the diet, a second study in which both carcinogen and benzyl isothiocyanate are given by oral intubation, a third study in which the carcinogen is given by oral intubation and the benzyl isothiocyanate is given intraperitoneally, a fourth study in which the carcinogen is given intraperitoneally and the benzyl isothiocyanate by oral intubation, and finally two studies in which the carcinogen

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is given intravenously. In one the benzyl isothiocyanate will be given by oral intubation and in the second it will be given intraperitoneally. In the third animal model, BP will be administered by the intratracheal route employing a technique comparable to that described by Ho and Furst (16). Benzyl isothiocyanate will be given by oral intubation, i.v. or i.p. The fourth model that will be employed will be that of DMBA-induced mammary tumor formation. Previous work has shown that benzyl isothiocyanate given either i.p. or by oral intubation prior to administration of DMBA by oral intubation, will result in inhibition of mammary tumor formation. We propose to carry out modifications of this procedure in which the carcinogen is given intravenously instead of by oral intubation and the benzyl isothiocyanate either by oral intubation or i.p..

Studies of inhibition by benzyl isothiocyanate of carcinogenesis from carcinogens other than polycyclic aromatic hydrocarbons. In these studies we propose initially to use A/HeJ mice employing conditions found most effective for inhibiting pulmonary adenoma formation in the studies described above. Work will be carried out with several carcinogens. These include diethylnitrosamine, urethane, uracil mustard and 4-nitroquinoline-N-oxide. These studies will indicate whether inhibition by benzyl isothiocyanate occurs with a variety of carcinogens other than polycyclic hydrocarbons and including one carcinogen; namely, uracil mustard, which is a direct alkylating agent. Depending on the results obtained, considerations will be given to work with other test systems and carcinogens. In addition significant inhibitors identified in the final section of this proposal will be studied against a variety of carcinogens in a manner comparable to benzyl isothiocyanate.

Dose-response studies. On the basis of the findings of the initial portion of the animal work with the four animal model systems, dose-response studies will be carried out to

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determine the capacity of various dose levels of benzyl isothiocyanate to inhibit BP carcinogenesis in several experimental systems. When these data become available consideration will be given to carrying out further work involving other carcinogens.

Studies of the relationships between chemical structure and carcinogen-inhibiting activity of compounds related to benzyl isothiocyanate. We propose to determine the inhibitory capacities of several other isothiocyanates against model systems described above using conditions which appear to be the most appropriate for comparative studies. The compounds to be employed will be of two types. The first are compounds which occur naturally and accordingly could have some impact on response to chemical carcinogens. These include phenylethyl isothiocyanate, p-hydroxybenzyl isothiocyanate and allyl isothiocyanate. In addition to these naturally occurring compounds, we propose to study several compounds related to benzyl isothiocyanate in which there is a varying ease of hydrolysis of the isothiocyanate group. One of the possibilities is that when the benzyl isothiocyanate enters the cell, the isothiocyanate group is hydrolyzed off and that this is the effective component. A second possibility is that the intact molecule is required for inhibitory activity. In order to investigate this question we propose to employ phenyl isothiocyanate, a compound in which the isothiocyanate group will not hydrolyze and also a series of para-substituted derivatives of benzyl isothiocyanate with differing electron withdrawing or donating activity i.e. CH_3O, CH_3S, CH_3, Cl and NO_2. An additional compound related to benzyl isothiocyanate that we propose to study is benzyl nitrile. Nitriles are also naturally occurring components of plants. Studies which have been carried out have shown that some nitriles have the capacity to inhibit carcinogenesis resulting from administration of nitrosamines. Accordingly benzyl nitrile would be of interest to study under comparable conditions to those

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employed for benzyl isothiocyanate. Depending on the results obtained with the above experiments additional compounds will be studied which have the potential of providing further information on inhibitory capacities of naturally occurring compounds, structural characteristics giving maximum inhibition and also those possibly giving insight into the mechanism of inhibition.

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General Bibliography

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11. Huberman, E., Kuroki, T., Marquardt, H., Selkirk, J.K., Heidelberger, C., Grover, P.L., and Sims, P.: Cancer Res. 32:1391-1396, 1972.

12. Jerina, D.M., and Daly, J.W.: Science 185:573-581, 1974.

13. Nagata, C., Inomata, M., Kodama, M., and Tagashira, Y.: Gann 59:289-298, 1968.

14. Cavalieri, E., and Calvin, M.: Proc. Nat. Acad. Sci. U.S. 68:1251-1253, 1971.

15. Wattenberg, L.W.: J. Natl. Cancer Inst. 48:1425-1430, 1972.

16. Ho, W., and Furst, A.: Oncology 27:385, 1973.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Three large laboratories and ample animal quarters under the direction of the principal investigator are available for use in this project. The laboratories are equipped with facilities for carrying out work in biochemistry and organic chemistry. They contain the following equipment: Liquid chromatograph (Waters Associates), Packard Tri-Carb liquid scintillation spectrometer 2425, Packard Model 420 gas chromatograph, Gilson linear fraction collector, Aminco-Bowman spectrofluorophotometer, Beckman Model J-21B centrifuge, Servall refrigerated centrifuges, balances, pH meters, and other equipment for biochemical work. A walk-in cold room, refrigerators and deep freeze (-50°C) units are present. Equipment for carrying out organic syntheses and purification of compounds is present. Equipment for carrying out routine and special histologic staining techniques is in the laboratories. In addition to our own equipment, the Pathology Department has shared equipment including a Beckman LS-250 Liquid scintillation counter. Specialized equipment for use in determining chemical structure such as nuclear magnetic resonance spectrographs and mass spectrometers are available for our use in the Department of Chemistry.

11. Additional facilities required:

None.

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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BIOGRAPHICAL SKETCH

NAME Lee W. Wattenberg, M.D. TITLE Professor of Pathology

BIRTH DATE REDACTED PLACE OF BIRTH REDACTED

NATIONALITY REDACTED

EDUCATION

College of the City of New York, New York	B.S.	1941	Premedicine
University of Minnesota, Minneapolis	B.M.	1949	Medicine
University of Minnesota, Minneapolis	M.D.	1950	Medicine

HONORS AND PROFESSIONAL ASSOCIATIONS

Alpha Omega Alpha
Sigma Xi
Borden Award Undergrad. Medicine Research
Lederle Medical Faculty Award 1956-59
President, American Histochemical Society 1966-67
Associate Editor, Cancer Research

RESEARCH AND PROFESSIONAL EXPERIENCE

Professor of Pathology - University of Minnesota	1966 - present
Associate Professor of Pathology - University of Minnesota	1959 - 1966
Visiting Scientist - Chester Beatty Research Institute	1963 - 1964
Assistant Professor of Pathology - University of Minnesota	1957 - 1959
Instructor, Department of Pathology - University of Minnesota	1955 - 1957
Medical Fellow - Department of Pathology - University of Minnesota	1954 - 1955
U.S. Army - 1st Lt. - (Dr. Joseph Smadel) Virus and Rickettsial Disease Department - Walter Reed Medical Center	1952 - 1954
Research Fellow, American Cancer Society - University of Minnesota Departments of Biochemistry and Pathology	1950 - 1952
Internship, Department of Medicine - University of Minnesota	1949 - 1950
Jr. Biologist, Manhattan Project, Medical Research Group	1944 - 1946

SELECTED BIBLIOGRAPHY

1. Alterations in Microsomal Metabolism of Benzo(a)pyrene in Mice by Butylated Hydroxyanisole. Jennine L. Speier and Lee Wattenberg. J. Natl. Cancer Inst. 54 1975 - (Preprint enclosed).

2. Aryl Hydrocarbon Hydroxylase Induction in Rat Tissues by Naturally Occurring Indoles of Cruciferous Plants. William D. Loub, Lee W. Wattenberg and David W. Davis. J.Natl. Cancer Inst. 54, 1975 (Reprint enclosed).

3. Inhibition of Carcinogenic and Toxic Effects of Polycyclic Hydrocarbons by Several Sulfur-Containing Compounds. Lee W. Wattenberg. J. Natl. Cancer Inst. 52:1583-1587, 1974 - (Reprint enclosed).

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4. Inhibition of Chemical Carcinogen Induced Pulmonary Neoplasia by Butylated Hydroxyanisole. Lee W. Wattenberg. J. Natl. Cancer Inst. 50:1541-1544, 1973 (Reprint enclosed).

5. Inhibition of Carcinogenic and Toxic Effects of Polycyclic Hydrocarbons by Phenolic Antioxidants and Ethoxyquin. Lee W. Wattenberg. J. Nat. Cancer Inst. 48:1425-1430, 1972 - (Reprint enclosed).

NAME Peter Borchert

TITLE Senior Scientist

BIRTHDATE REDACTED

PLACE OF BIRTH REDACTED

NATIONALITY REDACTED

EDUCATION

TH-Darmstadt College - Germany (Graduate School) Universität Heidelberg - Heidelberg, Germany	B.S	1964	Chemistry
	M.S.	1968	Chemistry
University of Wisconsin - Madison, Wisconsin	Ph.D.	1972	Oncology

RESEARCH AND PROFESSIONAL EXPERIENCE

University of Minnesota - Department of Laboratory Medicine & Pathology - Senior Scientist	1973 - present
McArdle Laboratory - Madison, Wisconsin - Post-doctoral Fellow	12/1972 - 9/1973
University of Wisconsin - McArdle Laboratory - Madison, Wisconsin Graduate Student	6/1968 - 11/1972
Universität of Heidelberg - 69 Heidelberg, Germany Graduate Student	10/1964 - 5/1968

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1. The Metabolism of the Naturally-occurring Hepatocarcinogen Safrole to 1'-Hydroxysafrole and the Electrophilic Reactivity of 1'-Acetoxysafrole. Borchert, P., Wislocki, P.G., Miller, J.A., & Miller, E.C. Cancer Research 33: 575-589, (1973).

2. 1'-Hydroxysafrole: A Proximate Carcinogenic Metabolite of Safrole in the Rat and Mouse. Borchert, P., Miller, J.A., Miller, E.C., and Shires, T.K. Cancer Research 33:590-600, (1973).

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Dr. Lee Wattenberg	15	----
Dr. Peter Borchert (Senior Scientist)	50	REDACTED
Technical		
Allan Fladmoe (Junior Scientist - Chemist)	100	
Junior Scientist - with credentials for animal work (to recruit)	100	REDACTED
Animal Caretaker (to recruit)	100	9,112
Fringe Benefits (17%)		7,827
Sub-Total for A		53,867
B. Consumable supplies (by major categories)		
Purchase and care of experimental animals		6,000
Purified diets		2,000
Chemicals		4,000
Glassware and misc. small items of equipment		2,500
Isotopically labelled compounds*		7,000
*(^{14}C-benzyl isothiocyanate, ^{14}C-benzo(a)pyrene, ^{3}H-benzo(a)pyrene)		
Sub Total for B		21,500
C. Other expenses (itemize)		
Travel - to scientific meetings		900
Sub-Total for C		900
Running Total of A + B + C		76,267
D. Permanent equipment (itemize)		
Fraction collector holding vials for scintillation counter (Gilson Company)		3,000
Sub-Total for D		3,000
E. Indirect costs (15% of A+B+C)		11,440
Total request		87,707

15. Estimated future requirements.

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip	Indirect Costs	Total
(increased by 10% over previous yr)						
Year 2	59,507	21,500	800	0	12,271	94,078
Year 3 (increased by 10% over previous yr)	65,735	22,500	800	0	13,355	102,390

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16. Other sources of financial support

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Inhibition of Chemical Carcinogenesis by Anti-oxidants	USPHS CA-14146	90,000	2-1-73 to 2-1-76
Inhibition of Neoplasia of the Large Bowel	USPHS CA-15638	143,300	1-1-74 to 12-31-76
Studies of Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	USPHS CA-33364 Contract	177,200	12-23-74 to 12-22-76
Histochemical & Quantitative Studies of Neoplasia	USPHS CA-09599	248,755	9-1-70 to 8-31-75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Inhibition of Chemical Carcinogenesis by Anti-oxidants	USPHS CA-14146 (renewal application)	503,402	2-1-76 to 1-31-81
Histochemical and Quantitative Studies of Neoplasia	USPHS CA-09599 (renewal application	473,780	9-1-75 to 8-31-80

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Dr. Lee W. Wattenberg

Signature Lee W. Wattenberg Date 5/29/75

Telephone 612 373-2808
Area Code Number Extension

Checks payable to

University of Minnesota

Mailing address for checks Mr. Clinton Johnson
Assistant Vice President
University of Minnesota
302 Morrill Hall
Minneapolis, MN 55455

Responsible officer of institution

Typed Name C. T. Johnson

Title Assistant Vice President

Signature C. T. Johnson Date June 4, 1975

Telephone 612 373-2058
Area Code Number Extension

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